NMR characterization of Streptococcus mutans WapA

Brotgandel, A. (UF, Biochemistry & Molecular Biology) Riviere, G.L. (UF, Biochemistry & Molecular Biology); Perera L. (UF, Oral Biology), Barran-Berdon, A. (UF, Oral Biology); Brady J. (UF, Oral Biology); and Long, J.R. (UF, Biochemistry & Molecular Biology)

Introduction

Streptococcus mutans has been shown to be the main pathogen responsible for human dental caries. Wall protein A (WapA) is an important contributor to the virulence of *S. mutans* because it enables the pathogen to anchor to the surface of teeth without the presence of sucrose. One of the naturally occurring derivatives of WapA is Antigen A (AgA), which is important to amyloid fibril formation in *S. mutans*, and it is considered a potential target for biofilm inhibition and the prevention of dental caries. Through an improved understanding of the structure of AgA, its mechanism of biofilm formation can be better understood and potential treatments for dental caries may be identified.

Experimental

For NMR experiments, we prepared U–[^{15}N]–AgA sample using a protocol developed by Marley et al [1]. The AgA protein was purified with affinity, ion exchange, and size exclusion chromatography. The AgA purification protocol needs to be further optimized to obtain a pure protein (Fig 1), but purity was sufficient for initial NMR characterization. Preliminary NMR ^{1}H-^{15}NHSQC experiments were performed at 23, 27, 30°C in order to study the stability of AgA using a Bruker Avance 600 instrument in the AMRIS Facility.

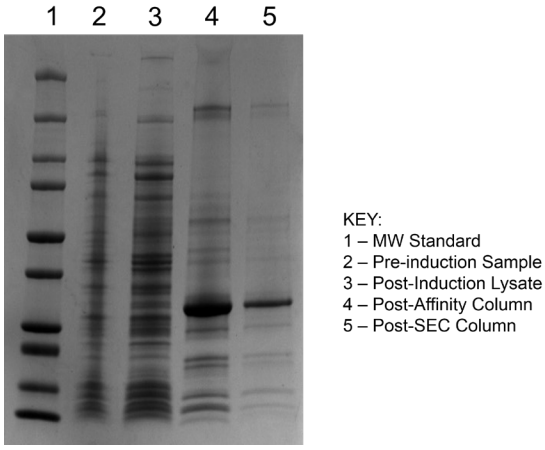

Fig 1: After two-step purification, the sample was still not pure, as shown by bands in the left-most column of the SDS-PAGE.

Results and Discussion

Preliminary data indicates that Aga is a folded protein (Fig 2) and stable at 23, 27, and 30 °C. We observed 280 ^{1}H-^{15}N resonances in the HSQC. In the future, we would like to identify quaternary interactions of AgA with cell wall preparations or the Adhesin P1 protein. For that, we will collect NMR titrations on ^{15}N-enriched AgA. It will be necessary to have the NMR assignments for AgA and a purer AgA sample.

Conclusions

Further optimization of production and purification protocol to obtain pure AgA is necessary. This will be used for the structural and functional characterization of AgA by NMR and other biophysical techniques. This is the first time AgA has been studied by NMR.

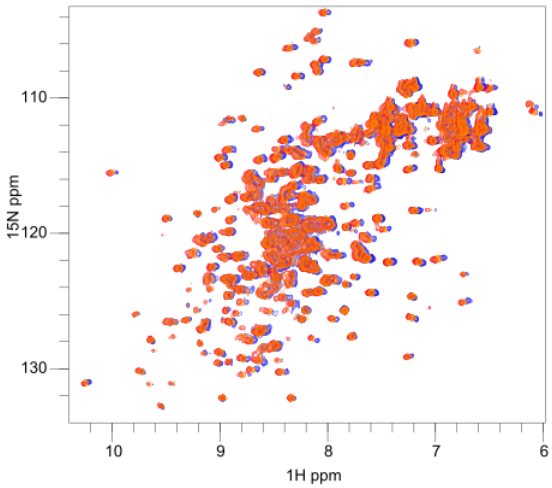

Fig 2: ^{15}N-^{1}H HSQC of Antigen A at 23°C (orange), 27°C (blue), and 30°C (red)

Acknowledgements

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References

[1] Marley J, et *al*., Journal of Biomolecular NMR, **20**, 71–75 (2001)